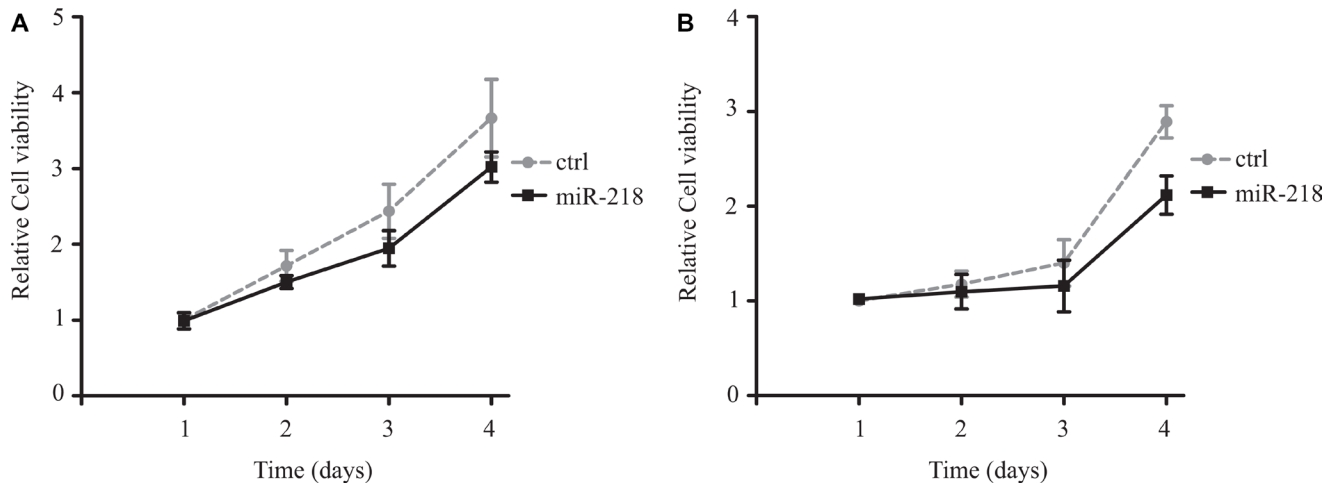
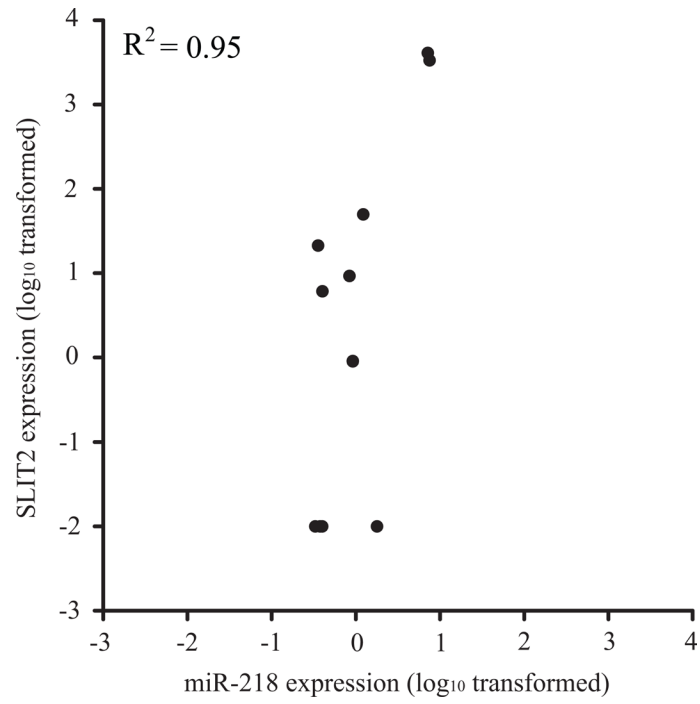


## MACC1 is post-transcriptionally regulated by miR-218 in colorectal cancer

### Supplementary Materials



**Supplementary Figure S1: Effect of miR-218 on cell viability of SW480 and MKN-45 cells.** SW480 (A) or MKN-45 (B) cells were transfected either with control-miR (ctrl) or miR-218 and cell viability were determined using the colorimetric MTT assay. Absorption at 560 nm of day 0 was used for seeding normalization. Data are represented as mean  $\pm$  SEM of their biological replicates, each experiment with three technical replicates.



**Supplementary Figure S2: SLIT2 and miR-218 expression in log<sub>10</sub> scale.** Expression data of all 11 cell lines included in the correlation analysis presented as log<sub>10</sub> transformed scales.

**Supplementary Table S1: Patients information**

		Total	Percentage
Sex	M	36	61%
	F	23	39%
Age median (min to max)	63.0 (37.9 to 87.8)		
UICC (7. Edition)	I	17	29%
	II	22	37%
	III	20	34%
pT	1	6	10%
	2	16	27%
	3	27	46%
	4	10	17%
pN	0	39	66%
	1	14	24%
	2	6	10%
Metachronous metastases	no	35	59%
	yes	24	41%

Clinico-pathological features of the 59 consecutively resected CRC patients. pT = Pathologic tumor classification. pN = Pathologic lymph node status.

**Supplementary Table S2: Oligos used for MACC1-3'-UTR cloning**

No	Oligo Name	Sequence (5'---3')
1	MACC1_UTR_For	AGGGAGCTCTTGATGGGAGGGAAAATGAG
2	MACC1_UTR_Rev	ATTCTCGAG TTCAGCTTGCCACCTCTTCT

**Supplementary Table S3: Oligos used to mutate miR-218 seed sequences at MACC1-3'-UTR**

No	Oligo Name	Sequence (5'---3')
1	M3U_Mut_For	CAGTTTTTTTTTTCCTGATGAAAGCTGTGCCTAACTGATAACCAAGATGGGT
2	M3U_Mut_Rev	ACCCATCTTGGTTATCAGTTAGGCACAGCTTTCATCAGGAAAAAAAAAACTG

**Supplementary Table S4: Oligos used for sequencing the MACC1-3'-UTR**

No	Oligo Name	Sequence (5'---3')
1	MACC1_Seq_1	GGAAACAGTTGCCGTTTCAT
2	MACC1_Seq_2	AGTTGCAATGGAGAGGCTGT
3	MACC1_Seq_3	CTTGCAGTGAGCCGAGTTC
4	MACC1_Seq_4	CCTTGTCGCGGTAATTTTTG
5	MACC1_Seq_5	GCATTGGGCTGAGAAACACT
6	MACC1_Seq_6	TGGTCCACAGCACAAAATAAA
7	MACC1_Seq_7	AAGGCATAGCACATTTAGCACA

**Supplementary Table S5: Oligos used for MACC1 and RPII screening by qRT-PCR**

No	Gene Name	Sequence (5'---3')
1	MACC1_For	TTCTTTTGATTCTCCGGTGA
2	MACC1_Rev	ACTCTGATGGGCATGTGCTG
3	RPII_For	GCACCACGTCCAATGACAT
4	RPII_Rev	GTGCGGCTGCTTCCATAA

**Supplementary Table S6: Oligos used for methylation-specific PCR of MACC1, SLIT2 and SLIT3**

No	Gene Name	Sequence (5'---3')
1	SLIT2_M_F	GGGAGGCGGGATTGTTTAG
2	SLIT2_M_R	CATAACGCGCGAAAATACAC
3	SLIT2_U_F	GTGGGAGGTGGGATTGTTTA
4	SLIT2_U_R	ACCTCTCCCTCACCTCAAC
5	SLIT3_M_F	GGTTTCGTCGATGGAGTTGT
6	SLIT3_M_R	AAACGCGTAAAACCCGAAA
7	SLIT3_U_R	TGTGGGTTAGTGGGGTTAGG
8	SLIT3_U_R	CACAAACAAAACAAAACACTCCA

**Supplementary Table S7: Oligos used for generating cDNA of different poly-A tails in MACC1 mRNA and amplification of alternative polyadenylation (APA) sites**

No	Oligo Name	Sequence (5'---3')
1	Oligo(dT) Primer	GCGCATCGATCGGCCGCGTTTTTTTTTTTTTTTTV
2	Anchor Primer_R	GCGCATCGATCGGCCGCG
3	First_APA_F	TGT GGG TAA GCC AAA GAA GG
4	Second_APA_F	CAGCATGAAAGGCTTCCTCT
5	Third_APA_F	CCAAACATGCTCCAGTCTCA
6	Fourth_APA_F	TGCCCTGCTTTCACTGTAGA